332

MYCOBACTERIAL INFECTIONS IN ADULT SALMON AND STEELHEAD TROUT RETURNING TO THE COLUMBIA RIVER BASIN AND OTHER AREAS IN 1957

SPECIAL SCIENTIFIC REPORT–FISHERIES No. 332

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Explanatory Note

The Special Scientific Report series comprises results of investigations, usually of restricted scope, intended to aid or direct management or utilization practices and as guides for administrative or legislative action. Reports in this series are issued in limited quantities for the official use of Federal, State, or other cooperating agencies, and in processed form for economy and to avoid delay in publication.

United States Department of the Interior, Fred A. Seaton, Secretary
Fish and Wildlife Service, Arnie J. Suomela, Commissioner
Bureau of Sport Fisheries and Wildlife, Daniel H. Janzen, Director

MYCOBACTERIAL INFECTIONS IN ADULT SALMON AND STEELHEAD TROUT RETURNING TO THE COLUMBIA RIVER BASIN AND OTHER AREAS IN 1957

By

A. John Ross[1/], Brian J. Earp[2/], and James W. Wood[3/]

Special Scientific Report--Fisheries No. 332.

1/ Bureau of Sport Fisheries and Wildlife, Seattle, Wash.
2/ Washington State Department of Fisheries, Seattle, Wash.
3/ Oregon Fish Commission, Oakridge, Oregon

Washington, D. C.

December 1959

C O N T E N T S

	Page
Mycobacteria in adult salmonid fishes returning to Federal hatcheries in Washington, Oregon and California, by A. John Ross	1
Mycobacteria in adult salmonid fishes returning to State hatcheries in Washington, by Brian J. Earp	10
Addendum	13
A survey of tuberculosis in Pacific salmon and steelhead trout in Oregon streams in 1957, by James W. Wood	18

INTRODUCTION

A survey of the diseases of Columbia River salmon and steelhead trout was initiated in 1957 in joint recognition that fish diseases are a major factor influencing the successful maintenance and rehabilitation of salmon and steelhead populations in the Columbia River Basin and neighboring regions. Agency responsibility was delineated and contracts for the work let by the U. S. Fish and Wildlife Service to the Washington Department of Fisheries and Oregon Fish Commission.

Initial studies were limited to an appraisal of the geographical distribution, incidence, and intensity of the disease agent responsible for the tuberculosis-like infection of salmonid fishes. It was deemed that an understanding of the infection would guide management practices with subsequent control of the infection substantially reducing losses in salmon and steelhead populations.

Each of the participating agencies compiled the results of its 1957-1958 study in a manner most appropriate to the data obtained. The reports of each agency are presented here as submitted.

MYCOBACTERIA IN ADULT SALMONID FISHES RETURNING TO FEDERAL HATCHERIES IN WASHINGTON, OREGON AND CALIFORNIA

By

A. John Ross
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

ABSTRACT

The degree of incidence of acid-fast bacillus infections in adult salmonid fishes was determined. The disease was shown to be widely distributed in the area examined. It is believed the primary source of infection is derived from the hatchery practice of feeding infected salmon products to juvenile fish. One group of marked adults that had been hatchery reared for 370 days showed a 62 percent incidence of infection. A statistical analysis indicated that length of fish is independent of infection.

Earp, Ellis and Ordal (1953) reported the presence of acid-fast bacilli in chinook salmon from the Columbia River. The geographical range of the disease agent has been extended to include California, Oregon, Washington and Alaska. During the ensuing years, Wood and Ordal (1959) and Ross (1959) identified bacteria of similar nature from a wide spectrum of host species belonging to the Salmonidae. In view of the widespread distribution of this fish pathogen, a survey was initiated in 1957 by the U. S. Fish and Wildlife Service with the Washington State Department of Fisheries and the Oregon State Fish Commission cooperating under contract. It was conjectured that the data resulting from such a survey might cast some light upon the effects of the disease with respect to species of fish infected, size of fish and increased susceptibility to the disease of either sex. In addition, it was recognized that significant differences in incidence by hatchery or geographical location might aid in determining the sources of the infection.

It has been recognized that the feeding of infected salmon products to hatchery-reared juveniles may well be the primary method of disseminating the micro-organisms. At the present time, very little is known of the role played by vectors in the water sources, or of the microflora of water itself.

METHODS

Whole livers from adult salmonids returning to Federal egg taking stations in Washington, Oregon and California were collected by personnel of the U. S. Fish and Wildlife Service. Additionally a number of rivers representing natural spawning areas was sampled.

In most instances, livers were placed in individual cellophane bags as they were removed from the fish, and were frozen prior to delivery to the Western Fish Disease Laboratory. Material from three separate regions of each liver was smeared on glass micro-slides following thawing of the samples. These smears were stained by the differential method of Ziehl-Neelsen, and each slide examined with an oil immersion objective for five minutes. Slides showing the presence of acid-fast bacilli were given a numerical rating of 1 to 5 depending upon the number of organisms present (table 2).

Chi square values (Siegel 1956) were

computed in order to determine if a relationship existed between infection and length of fish.

RESULTS

Natural spawning areas

Livers were examined from chinook salmon spawning under natural conditions in each of 17 rivers in Washington. No incidence of infection was found (table 1).

Results obtained from the examination of sockeye salmon spawning in the Okanagon River are shown in table 2. Although salmon found in the Okanagon River are presumed to be wild stocks, the possibility of strays from hatchery stocks entering the river must be given consideration.

Artificial spawning areas

Little White Salmon Station

Livers from fall chinook salmon were collected and divided into three groups: random sample, marked fish and grossly diseased or abnormal fish. The random sample was subdivided by sex and the results of the examination are shown in tables 3 and 4. X^2 values indicated the infection was randomly distributed in the female population with respect to length class, however, the proportion of infected fish in each size stratum was not equal in the males. A X^2 value of 14.98 was calculated from the data which is significant at the .01 level with 2 degrees of freedom. It is possible this group was composed of two age-classes with the higher incidence of infection occurring in the older fish.

Sixty-three livers were obtained from grossly abnormal fish. Fish were considered to deviate from the normal if lesions were present on the liver, kidney or spleen. In addition, fish showing off coloration, retardation of growth and/or underdeveloped gonads were also considered abnormal. Acid-fast bacilli were present in 100 percent of this sample. Thirty-eight fish marked with fin clips returned to the hatchery with 22 being infected (table 5).

Carson Station

Livers were collected from 387 spring chinook salmon trapped at Bonneville Dam and held at the Carson station (tables 5, 6 and 7). In contrast to the results obtained at the Little White Salmon station the female upstream migrants held at this hatchery appeared to be composed of two age-classes; however, the larger fish again showed a higher incidence of infection with a calculated X^2 value of 14.72 being significant at the .01 level with 3 degrees of freedom. Infections appeared to be randomly distributed between length-classes in the male population.

Entiat Station

Summer chinook and sockeye salmon were taken at the Entiat hatchery. Due to the relatively small number of fish available of each species, no attempt was made to compare the samples by sex. The X^2 value was computed for each species only, and the incidence of infection was determined to be proportionate to the length class for both groups (tables 8 and 9).

In a number of instances either no infection was present, or complete data was unavailable. Percent infection rates are tabulated for this group in table 10.

DISCUSSION

Due to the varied procedures and availability of personnel at different stations, there is some variation in the resulting data. Information regarding lengths and sex was not available for all fish from which livers were obtained; therefore, direct comparisons cannot be made at all hatcheries.

Analysis of length versus incidence of infection revealed significant differences in two of six samples. The two groups consisted of male fall chinook salmon from the Little White Salmon station and female spring chinook from the Carson station. In both instances, however, length-frequency curves indicated the possible presence of 2 year-classes in the sample. A

higher incidence of infection was found in the larger (or older) fish of both groups. This finding is consistent with the chronic nature of the disease. If it is assumed that the disease is contracted through hatchery feeding practices, it is not unreasonable to further assume that the micro-organisms may be present in younger fish; however, the rate of multiplication and subsequent organ involvement is such that these bacteria are more readily discernible in older fish and may be overlooked in younger fish with a lesser degree of infection. As an alternative, it might be speculated that a difference in feeding practices between brood years may also contribute to a difference in incidence of infection when two or more age-classes are encountered in a sample. It appeared, however, that within the same age group no relationship existed between length of fish and infection. In addition, it has been shown that there was no relationship between infection and sex.

The diets at all hatcheries, with the exception of the Eagle Creek installation, contained salmon products during the rearing of the 1953-54-55 broodstocks. Therefore, all hatchery reared salmon collected under the survey program had presumably been fed some salmon products. It is obvious, however, that all lots of salmon products are not necessarily infected with acid-fast bacilli or even infected to the same degree; thus, some stations may be free of the disease, or relatively so, despite the use of diets containing salmon products. One very important factor in the spread of the disease in hatchery-reared fish may be related to the length of time fingerlings are reared prior to release. This may be demonstrated in the results obtained from the Little White Salmon station marked fish studies (table 5). Marked fish returning to this station were from the 1953 brood year, reared and released at the Little White Salmon station. Thirty-five fish (22 infected) fin-marked An-RP were from a release of 109,000 yearlings reared for 370 days. Five non-infected fish fin-marked D-RV were from a release of 224,000 fingerlings reared for 112 days. As the primary source of the acid-fast organisms is considered to be infected salmon products used for food, it may be postulated that the 62 percent rate of infection among the returning fish reared for 370 days is due to the extended period of time this group had been subjected to contaminated food. As the incidence of recovery of marked fish is also greater in the fish reared for the longer period, it may be further postulated that the majority of unmarked fish showing grossly diseased livers might also have been reared over the longer period of time. Conclusive evidence must, however, be derived from a larger sample. It is of interest to note that although both groups of marked fish were of equal length, the five fish reared for 112 days averaged three pounds more in weight.

It is suspected from the results of this study that in the same age-class no relationship existed between length of fish and infection. No information is presented that would indicate a species resistance or an increased susceptibility to infection of either sex. The high percentage of infected fish found in presumably wild stocks from the Okanagan River is of extreme interest. Acid-fast bacilli rarely have been found in wild fish populations and an infection rate of 36 percent in the Okanagan River is startling indeed. Unfortunately, however, it cannot be definitely stated that these fish are not hatchery strays.

Definite evidence regarding the appearance of acid-fast bacilli in wild stocks of salmon is presented in the results of a 1958 survey of downstream migrant sockeye fingerlings leaving Redfish Lake in Idaho. Two of 368 fish examined were infected with acid-fast bacteria and there is no evidence that this lake supports anything but wild populations.

It may be of interest at this point to consider the findings from the examination of approximately 1,000 slides prepared from livers of salmon obtained in 1955 from Alaskan waters. Acid-fast bacilli were noted in three adult sockeye salmon from this group. Two of the infected fish were taken about 75 miles west of Juneau while the third specimen was netted in Lake Cories on Attu Island. Again, it may only be strongly suspected that these fish were of wild origin.

Due to the technical limitations of the staining and examining procedures used in this study, it is believed that the actual number of infected fish is greater than indicated herein.

ACKNOWLEDGMENT

The author wishes to thank Harland Johnson, Fishery Biologist, Little White Salmon station, and other personnel of the Fish and Wildlife Service who aided in the collection of the sample material.

LITERATURE CITED

Earp, B. J., C. H. Ellis, and E. J. Ordal
1953. Kidney disease in young salmon. State of Washington Department of Fisheries, Special Report Series No. 1.

Ross, A. J.
1959. Mycobacterium salmoniphilum sp. nov. from salmonid fishes. Am. Rev. Resp. Dis. In press.

Siegel, S.
1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co., Inc., New York.

Wood, J. W., and E. J. Ordal
1959. Tuberculosis in Pacific salmon and steelhead trout. Contributions of The Fish Commission of the State of Oregon, No. 25.

Table 1.--Incidence of acid-fast bacilli in chinook salmon spawned under natural conditions in Washington rivers

Source	Number examined	Number infected
Little Wenatchee River	2	0
Early Winters Creek	3	0
Chewack River	11	0
Lake Creek	3	0
Lost River	3	0
Entiat River	10	0
American River	11	0
Nason Creek	8	0
Naches River	5	0
Twisp River	19	0
Wenatchee River	10	0
Yakima River	9	0
Similkameen River	3	0
Methow River	30	0
Bumping River	6	0
Little Naches River	6	0
Chiwawa River	13	0

Table 2.--Degree in incidence of acid-fast bacilli infections in sockeye salmon spawned under natural conditions in the Okanagan River

Degree of infection*	Sex		Average length in inches
	Males	Females	
0	16	16	19.8
1	3	7	
2	3		
3	2	1	19.6
4		1	
5		1	
Total No. of observations	24	26 = 50	
Total No. infected	8	10 = 18	

Percent infection = 36

*1.--one to 20 organisms per slide
2.--over 20 organisms per slide
3.--one to 25 organisms per field
4.--25 to 150 organisms per field
5.--over 150 organisms per field

(each slide examined five minutes)

Table 3.--Degree of incidence of acid-fast bacilli infections in female fall chinook salmon collected at random at the Little White Salmon station

Degree of infection	Length of fish in inches			
	28 - 31	32 - 35	36-39	40 - 45
0	5	40	85	15
1		2	3	
2	1		2	
3			4	2
4			3	
5		1	3	1
Total No. of observations	6	43	100	18=167
Total No. infected	1	3	15	3=22

Percent infected = 11.2

Table 4.--Degree of incidence of acid-fast bacilli infections in male fall chinook salmon collected at random at the Little White Salmon station

Degree of infection	Length of fish in inches						
	18-21	22-25	26-29	30-33	34-37	38-41	42-45
0	16	21	14	23	53	45	3
1					3	3	
2						1	
3					3	2	
4					2	2	
5					3	2	1
Total No. of observations	16	21	14	23	64	55	4=197
Total No. infected	0	0	0	0	11	10	1=21

Percent infected = 13.2

Table 5.--Degree of incidence of acid-fast bacilli infections in abnormal* and marked** fall chinook salmon collected at the Little White Salmon station

Degree of infection	abnormal	Mark An-RP	Mark D-RV
0		13	5
1	5	6	
2		2	
3	4	7	
4	3	2	
5	51	5	
Total No. of observations	63	35	5
Total No. infected	63	22	0
Percent infected	100	62	0

* Average length = 34 inches

** Average length of both infected and non-infected groups = 31 inches

Table 6.--Degree of incidence of acid-fast bacilli infections in female Spring chinook salmon collected at random at the Carson station

Degree of infection	Length of fish in inches					
	23-26	27-30	31-34	35-38	39-42	43-46
0	10	101	45	34	3	1
1	2	4	4	12		
2						
3		1				
4						
5						
Total No. of observations	12	106	49	46	3	1=217
Total No. infected	2	5	4	12	0	0=23

Percent infected = 10.6

Table 7.--Degree of incidence of acid-fast bacilli infections in male Spring chinook salmon collected at random at the Carson station

Degree of infection	18-21	22-25	26-29	30-33	34-37	38-41	42-45
0	1	16	73	33	9	8	2
1	1	2	13	7	1	1	1
2							
3		1	1				
4							
5							
Total No. of observations	2	19	87	40	10	9	3 = 170
Total No. infected	1	3	14	7	1	1	1 = 28

Percent infected = 16.2

Table 8.--Degree of incidence of acid-fast bacilli infections in male and female sockeye salmon collected at random at the Entiat station

Degree of infection	15-16	17-18	19-20	21-22	23-24
0	4	2	45	38	3
1	1	1	5	4	
2			2		
3					
4					
5					
Total No. of observations	5	3	52	42	3 = 105
Total No. infected	1	1	7	4	0 = 13

Percent infected = 12.3

Table 9.--Degree of incidence of acid-fast bacilli infections in male and female summer chinook salmon collected at random at the Entiat station

Degree of infection	Length of fish in inches							
	13-16	17-20	21-24	25-28	29-32	33-36	37-40	41-44
0	5	20	8	6	10	25	9	2
1		1	2			4		1
2		1						
3								
4								
5								1
Total No. of observations	5	22	10	6	10	29	9	4 = 95
Total No. infected	0	2	0	0	0	4	0	2 = 8

Percent infected = 10.5

Table 10.--Percent of acid-fast bacilli infections in adult fish returning to federal hatcheries in Washington, Oregon and California

Hatchery		Total No. examined	Total No. infected	Percent infected
Coleman, Calif.	fall & spring chinook	93	20	21.5
Eagle Creek, Ore.	steelhead	22	0	0.0
Quilcene, Wash.	silvers	85	1	1.1
Leavenworth, Wash.	sockeye	117	0	0.0
Winthrop, Wash.	(summer chinook)	31	0	0.0
	(sockeye	107	1	0.9
Spring Creek, Wash.	fall chinook	300	7	2.3
Abnormal Spring Creek, Wash.	fall chinook	10	9	90.0

MYCOBACTERIA IN ADULT SALMONID FISHES RETURNING TO STATE HATCHERIES IN WASHINGTON

By

Brian J. Earp
With Addendum by R. L. Westgard
Washington State Department of Fisheries

ABSTRACT

A survey for tuberculosis in salmon returning to hatcheries of the State of Washington Department of Fisheries situated in the Columbia River Basin was conducted. Chinook salmon (Oncorhynchus tshawytscha) and silver salmon (Oncorhynchus kisutch) were examined for the presence of acid-fast bacteria. Tissues were examined by the standard bacteriological smear technique and by a method designed to concentrate the bacteria dispersed throughout the liver tissue.

The Columbia River Disease Task Force was formed in joint recognition that fish diseases are a major factor influencing the successful maintenance and rehabilitation of salmon and steelhead populations in the Columbia River Basin and that this area of study had been neglected in the past. The Department of Fisheries, with other fisheries agencies and universities of the States of Washington, and Oregon, and the U.S. Fish and Wildlife Service agreed in the necessity for increased emphasis on the problems of fish disease toward the end that their solution would guide management practices and substantially reduce losses from disease in salmon and steelhead populations. Greatest emphasis was given to determining the incidence of infections by acid-fast bacteria in the various runs and species of salmon and steelhead trout.

This report deals specifically with the survey of the incidence of acid-fast infections in salmon in the designated areas and with the results of that survey.

METHODS

The studies conducted during the fiscal year 1958 entailed the collection of whole livers from adult migrant salmon at hatcheries of the State of Washington Department of Fisheries situated in the Columbia River watershed, during the 1957 spawning season. These included Toutle, Elokomin, Kalama, Lewis River and Klickitat hatcheries. Liver samples were also taken from adult salmon at the Samish hatchery. This hatchery is located on the Samish River in northwest Washington and is representative of Washington State hatcheries lying outside the Columbia River watershed. Individual livers were preserved by freezing in plastic sacks and transported to the Department of Fisheries laboratory.

The liver samples were processed and examined by two different methods employed simultaneously on each sample; one by the standard bacterial smear method and the other by a concentration technique developed in our laboratory. In the standard smear method a small piece of liver tissue was smeared on a glass slide. In the concentration method, the entire liver was emulsified and the acid-fast bacteria concentrated by centrifugation and the resultant centrifugate smeared on a slide. The techniques of concentration of the acid-fast bacteria are dealt with in detail in the addendum to this report. In both methods the preparations were stained by the Ziehl-Neelsen method and examined microscopically for the presence of acid-fast bacteria. Slides were rated numerically depending upon the numbers of acid-fast bacilli present as follows:

No. 1 1-20 bacilli in entire preparation
No. 2 Over 20 bacilli in entire preparation
No. 3 1-25 bacilli per field
No. 4 25-100 bacilli per field
No. 5 Over 100 bacilli per field

RESULTS

The survey is incomplete insofar as samples were not obtained from all species normally found at the various hatcheries. For the purpose of this work only chinook salmon, Oncorhynchus tshawytscha and silver salmon, Oncorhynchus kisutch were considered since these two species are the only ones normally reared for any appreciable length of time at the hatcheries examined. At the Elokomin, Toutle and Lewis River hatcheries, samples of both fall chinook and silver salmon were obtained. At the Kalama, Klickitat and Samish hatcheries only fall chinook salmon samples were collected. Klickitat hatchery also receives a run of spring chinook salmon in addition to the fall chinook and silver salmon runs but no spring chinook salmon samples were obtained. No samples were collected from fish spawning naturally in this area which could be considered of "wild" stock as opposed to those reared artificially in hatcheries. Insufficient length-weight data were collected to be of significance.

The results of the survey for acid-fast bacteria are presented in table 1 by hatcheries and species. Results obtained by the direct smear and by the concentration method are listed separately.

DISCUSSION

Tuberculosis in salmon was first reported in adult chinook salmon returning to the Bonneville hatchery on the Columbia River by Earp, Ellis and Ordal (1953). It has since been postulated that the incidence of this disease in adult salmon might be connected, in some manner, to hatchery techniques, particularly the practice of feeding fresh frozen salmon products in the form of viscera and spawned out carcasses to young salmon as part of the hatchery diet. The feeding of salmon carcasses has been discontinued in the Columbia River Basin hatcheries by the State of Washington Department of Fisheries but frozen salmon viscera is still a major part of the hatchery diet.

Under normal hatchery procedures, fall chinook salmon are artificially reared for approximately 90 days from the time the fish start feeding and are then released to the stream for migration to the sea. Spring chinook salmon and silver salmon are reared for periods up to 12 months before being released in accordance with their normal migration pattern. Chum salmon (Oncorhynchus keta) and pink salmon (Oncorhynchus gorbuscha) migrate almost immediately to the sea after emerging from the gravel and are seldom reared artificially for any extended period in fresh water hatcheries. Only occasional small experimental lots of blueback salmon (Oncorhynchus nerka) are reared in Columbia River hatcheries by the State of Washington Department of Fisheries. Therefore, under normal hatchery conditions, chum and pink salmon are seldom artificially fed at these hatcheries, fall chinook are fed for approximately 90 days and spring chinook and silver salmon may be fed for periods of up to one year.

Considering the hypotheses that infection by acid-fast bacteria in salmon is introduced by the feeding of infected salmon products and that the incidence of infection is directly proportional to the length of time salmon are artificially reared (and exposed to infected foodstuffs), it could be expected that silver salmon and spring chinook salmon would show a higher incidence of infection than would fall chinook salmon. The results obtained in the survey at Elokomin and Lewis River hatcheries tend to bear out this hypothesis , particularly so in the case of Lewis River hatchery where incidence in the silver salmon was relatively high while the chinook salmon showed no incidence of the disease. At the Toutle hatchery, however, more chinook salmon than silver salmon were infected. Unfortunately the data are incomplete inasmuch as we have no samples from silver salmon at three of the hatcheries to compare with the incidence of infection in the chinook salmon.

Another incongruity was found in the case of the samples taken at the Elokomin hatchery which included both chinook and silver salmon. In both species taken at this hatchery a larger percentage of positive smears was detected by the direct smear method than was found by the concentration method. In all cases the acid-fast bacteria in the Elokomin samples proved to be severely damaged by the technique of concentration, retaining little if any of the typical morphology of acid-fast bacteria from samples taken at the other hatcheries. It is postulated that the bacterium found in the Elokomin fish differs in some respect from those found in fish from other locations and may represent an entirely different organism.

TABLE I

Hatchery	Species	No. Examined	Method	No. Positive	% Positive	Ratings (1)	(2)	(3)	(4)	(5)
Elokomin	Chinook	112	Direct Smear	5	4.5		1	2		2
			Concentration	4	3.6		1	3		
Elokomin	Silvers	181	Direct Smear	12	6.6	1	1	5	1	4
			Concentration	7	3.9	1	3	2		1
Kalama	Chinook	249	Direct Smear	12	4.8	7	3	1		1
			Concentration	10	4.0	6	3			1
Klickitat	Chinook	123	Direct Smear	2	1.6	2				
			Concentration	7	5.7	5	1	1		
Lewis River	Chinook	93	Direct Smear	0	0.0					
			Concentration	0	0.0					
Lewis River	Silver	102	Direct Smear	2	2.0	2				
			Concentration	18	17.6	2	10	5		1
Toutle	Chinook	238	Direct Smear	7	2.9	5	2			
			Concentration	9	3.8	3	6			
Toutle	Silver	110	Direct Smear	1	0.9		1			
			Concentration	1	0.9		1			
Samish	Chinook	26	Direct Smear	0	0.0					
			Concentration	0	0.0					

LITERATURE CITED

Earp, B. J., C. H. Ellis, and E. J. Ordal
1953. Kidney disease in young salmon. State of Washington Department of Fisheries, Special Report Series, No. 1.

A D D E N D U M

A PROCEDURE FOR THE DETECTION OF ACID-FAST BACTERIA IN FISH: A COMPARATIVE STUDY OF DIGEST-CONCENTRATE VS. SMEAR TECHNIQUE

By
Richard L. Westgard
State of Washington Department of Fisheries

ABSTRACT

A method has been developed to detect the presence of acid-fast organisms and to determine their relative number in fish tissue. This digest-concentrate method detects the presence of small numbers of organisms and those which are grouped in isolated tubercles which might escape detection with the standard smear technique. A comparative study was made between this digest-concentrate and the standard smear technique. Of 1,234 fish livers examined, 41 or 3.3 percent were positive by the smear method and 56 or 4.5 percent were positive by the digest-concentrate technique. This is a 36.4 percent increase in positives of the latter over the first. A combination of both methods gave a total of 77 positives or 6.2 percent of the number examined; a yield of 87.9 percent increase over the smear method and a 37.8 percent increase over the digest-concentrate.

In order to conduct a survey of the incidence of acid-fast bacteria in salmon and steelhead trout in the Columbia River Basin it was recognized that a more accurate and expedient method of detecting the presence of these bacteria in fish tissue would be desirable. This paper deals with such a method.

Acid-fast bacteria produce a systemic infection in salmon and steelhead trout with heavy concentrations of the organisms usually found in the liver. The liver was used exclusively for this study, because this tissue is conveniently obtained, handled and stored.

Gross observation and pathological studies of the diseased livers have shown that acid-fast bacilli tend to form in localized nodules instead of disseminating throughout the tissue. Unless gross infection is encountered, the bacilli would often be undetected by the usual methods of daubing small bits of tissue on a slide, or crushing tissue between slides. To overcome this the whole liver was liquefied producing an homogeneous distribution of bacteria. The concentration of an aliquot of the liquid yielded a known percentage of the total number of organisms per gram of liver by this method.

METHODS

Liquefaction

Various methods of liquefying the liver were tried including acid and enzymatic digestion. Heating for periods long enough to break down the liver tissue with HCl or H_2SO_4 destroyed the bacilli (1). Pepsin and trypsin gave only partial digestion (2). Treating with pepsin, decanting and treating the remaining undigested liver with trypsin gave about 90 percent digestion, but was costly and time consuming (3). Treating the livers with a 3-percent solution of NaOH was found to give approximately 95 percent digestion. This had no deleterious effects on the acid-fast bacilli as long as the temperature was kept below 52°C. However, at higher temperatures the bacilli began to disintegrate.

(1) Concentrations of HCl and H_2SO_4 tried: 3, 5 and 10 percent at 40°C., 60°C. and 100°C. for 10 min., 30 min. and 1 hr.

(2) Concentrations of pepsin tried: 0.25, 0.5 and 1.0 percent of 1:10,000 strength at 5ml./gm., 2ml./gm. and 1.5ml./gm. for 20 min., 30 min. and 1 hr. at 1 percent HCl and 52°C.
Concentrations of trypsin tried: 1, 5 and 10 percent of 1:250 strength at 5ml./gm., 2ml./gm. and 1.5 ml./gm. for 20 min., 30 min. and 1 hr. at pH 8.0-8.5 and 40°C.

(3) Concentration of pepsin used: 0.5 percent of 1:10,000 strength at 1.5/ml./gm. for 30 min. with 1 percent HCl at 52°C.
Concentration of trypsin used: 10 percent of 1:250 strength at 1.5ml/gm. for 30 min. at pH 8.0-8.5 and 40°C.

Laboratory procedure and preparation

Fresh livers were inserted in watertight plastic sacks and frozen. The frozen livers were then weighed, the tare weight of the plastic sacks subtracted and the sacked livers placed in appropriate beakers (table 1).

Table 1

Wt. of liver	Size of beaker
0-55 grams	150 ml.
56-90	250
91-100	400
151-230	600
over -230	800

If the sacks leaked after being frozen, the livers were placed in an additional watertight sack before further processing. For convenience, the weight of the liver was recorded on the beaker. The beakers were filled with hot tap water (45°C.-55°C.) without allowing the water to enter the plastic sacks and placed into a 45°C. water bath for a minimum of two hours. Thawing of the ice crystals aids in the breakdown of liver cells to prepare for final liquefaction; the faster the thawing, the more complete the breakdown of tissue.

Digestion

Water was poured out of the beakers and following the preparation of a smear slide for comparative purposes each liver was emptied from the sack into a beaker. Three percent NaOH was added in the quantity of 1.5 ml./gm. of liver. The mixture was stirred and put back in the water bath at 40°C. for approximately 16 hours after which 3 percent NaOH was added to volume, the water bath turned up to 43°C., and the livers stirred about every half hour for 2 to 3 hours.

Centrifugation

A 33 ml. aliquot of each liquefied liver was centrifuged for 30 minutes in a 55cc. hard plastic or stainless steel centrifuge tube at 16,000 rpm., or 34,800 G's. The addition of 10 mls. of a solution containing 9 percent Tween 80 in 95 percent ethyl alcohol before centrifuging inhibited the formation of a liquid layer on top which traps bacteria and would thus render the final bacterial count unsatisfactory.

If the centrifugation takes place in a cold room, approximately 12 ml. of 9 percent alcoholic Tween 80 should be used.

Smearing

The liquid was poured off and the tubes inverted on a paper towel to drain for two minutes. 1.5 mls. of distilled water were added to the centrifugate. In order to disperse the bacteria in the water, the mixture was stirred with a large glass stirring rod and allowed to sit a few minutes for the debris to settle. 0.05 ml. of the liquid was then pipetted onto each slide. The liquid was spread evenly encompassing an area of 5 sq. cm. This was most easily accomplished by tracing a 25-cent coin on the working surface, laying the slide over this outline, and spreading the liquid within the outline with a sterile inoculating loop. An ordinary 25-cent piece has an area extremely close to 5 sq. cm.

Staining and reading slides

The Ziehl-Neelsen technique proved satisfactory for staining large numbers of slides. Staining with fluorescent dye and observing under low power (20 X objective, 20 X ocular) with fluorescence microscopy is under investigation.

One square mm. of stained area was examined using the oil immersion objective. It was necessary to calibrate the microscopic field. A pattern of observation was established to cover as much of the 5 sq. cm. smear as possible. The use of wide-field eye pieces is unsatisfactory as the curvature of field inherent in all oil immersion objectives gives a hazy image at the periphery of the field and many organisms might be undetected.

Calculations

It was determined that for each organism seen in a square mm. there are approximately 1 million organisms per gram of liver. This figure was derived by the following procedure:

A measured quantity of liver was seeded with a known quantity of acid-fast bacilli. The liver was processed and examined by the complete digestion technique. The lowest bacterial density per gram of liver that would consistently give 1-10 organisms per sq. mm. of area examined was the final figure used.

A 257 gram chinook salmon liver was examined and found to be free of acid-fast bacilli by both digest and smear methods. After heat thawing, the liver was in a pulpy macerated state and could be stirred to give a homogeneous mixture. 100 grams of the thawed, mixed liver was used for the seeding and the remainder was examined for the presence of acid-fast bacilli.

A grossly infected liver was used for making a suspension of acid-fast bacilli. This liver was digested as outlined above; however, the material was filtered before centrifuging, the centrifugate was then suspended in water, filtered and centrifuged again. A final suspension showed a high concentration of organisms relatively free of liver debris.

In determining the bacterial density per ml. of the homogenate, a Breed count was employed to give the desired accuracy (4). As this technique depended upon a random distribution, the viscosity of the water had to be increased since the bacteria gathered toward the periphery of the area smeared. The addition of a few drops of blood alleviated this problem. One square mm. of the smear was systematically examined under oil immersion.

(4) $M.F. = \frac{10,000}{\text{area of field}} = \frac{10,000}{.0122 \text{ sq.mm.}} = 820,000$

(M.F.) (average No./field = No. organisms / ml.

(820,000) (21) = 17,220,000

6 ml. of this suspension was final amount seeded.

(6) (17,220,000) = 103,320,000/100 grams of liver or 1,033,200/ gm. of liver

RESULTS

The last 3 columns of table 2 shows the discrepancies between the 2 methods. The first of these three columns gives the number of positives found by smear method not correlating with those of the digest-concentrate method. For example, of the 4 positive Elokomin chinook liver found by digestion technique, 3 did not have a reciprocal positive slide from the 5 positive smear slides. Thus, from only one liver acid-fast bacilli

were found in both the smear and digest slides. Because of this factor, the total number of positives recorded with both methods is greater than with either method separately. This is shown in the last two columns, giving the number and percent of positive livers for both methods combined.

Out of a total number of 1,234 fish livers examined, 41 or 3.3 percent were positive by the smear method and 56 or 4.5 percent were found positive by the digest-concentrate technique. This was a 36.4 percent increase in positive of the latter method over the first. Of greater significance, however, was the combination of both methods with a total of 77 positives or 6.2 percent of the number examined. This is an increase of 87.9 percent over the smear method, and a 37.8 percent increase over the digest-concentrate procedure.

The smear slides, rated 1-5 with a completely arbitrary rating, could not be expected to have a high correlation with the digest-concentrate slides rated A-E which gives a reasonably accurate bacterial density count per gram. It can be noted, however, that within limits of very high or very low bacterial count there is a moderate degree of correlation. Part of the cause for this low correlation of bacterial count between the two systems lies in the characteristic of the acid-fast bacteria to group in nodules. If one nodule was selected when doing a smear slide it might appear that the liver was heavily infected and a rating of No. 5 would be given; however, this same liver might be rated as No. 1 or even negative if another part of the liver was examined.

Some of the hatcheries studied gave a reversal in expected trend of larger percentage of positives with the digest method. With Elokomin silvers, this was especially true. It might be postulated that there are different species, strains, or even cycles of acid-fast bacilli infecting these fish and some of the types will not withstand the rigorous physical treatment of the digest-concentrate technique.

Positive smears rated 1-5 after 5 minute examinations are as follows:

No. 1 Only 1-20 bacilli in entire preparation.

No. 2 In excess of 20 in entire preparation to less than 1/field.

No. 3 About 1/field to 25/field.

No. 4 About 25/field to 100/field.

No. 5 In excess of 100/field.

Positive digests rated A-E after examination of 1 sq. mm. of prepared area are as follows:

A. 1-10 bacilli/sq. mm. = 1-10 million/gram of liver.

B. 10-100/sq. mm. = 10-100 million/gram of liver.

C. 100-1,000/sq. mm. = 100 million-1 billion/gram of liver.

D. 1,000-10,000/sq. mm. = 1 billion/gram of liver.

E. 10,000-infinity/sq. mm. = 10 billion-infinity/gram of liver.

Table 2.--

Hatchery	Species	No. Examined	Smear-S Digest-D	No. Positive	Percent Positive	Ratings 1,A	2,B	3,C	4,D	5,E	Positives not Correlating	Total No. Positive Livers	Total Percent Positive Livers
Elokomin	Chinook	112	S	5	4.6		1	2		2	3	6	5.3
			D	4	3.6		1	3					
Elokomin	Silver	181	S	12	6.6	1	1	5	1	4	0	12	6.6
			D	7	3.9	1	3	2		1			
Kalama	Chinook	249	S	12	4.8	7	3	1		1	6	18	7.2
			D	10	4.0	6	3			1			
Klickitat	Chinook	123	S	2	1.6	2					2	9	7.3
			D	7	5.7	5	1	1					
Lewis River	Chinook	93	S	0	0.0						0	0	0.0
			D	0	0.0								
Lewis River	Silver	102	S	2	2.0	2					1	19	18.6
			D	18	17.6	2	10	5		1			
Toutle	Chinook	238	S	7	2.9	5	2				3	12	5.0
			D	9	3.8	3	6						
Toutle	Silver	110	S	1	0.9		1				0	1	0.9
			D	0	0.0								
Samish	Chinook	26	S	0	0.0						0	0	0.0
			D	0	0.0								
Total		1,234		41-S = 3.3 percent of No. examined 56-D = 4.5 percent of No. examined								77 = 6.2 percent of No. examined	

A SURVEY OF TUBERCULOSIS IN PACIFIC SALMON AND STEELHEAD TROUT IN OREGON STREAMS IN 1957

By

James W. Wood
Oregon Fish Commission

ABSTRACT

A survey for tuberculosis in anadromous salmonid fishes was conducted in Oregon streams in 1957. Fish for examination were collected from the commercial gill-net fishery landings on the Columbia River, at hatchery egg-collecting stations, and from fish of natural origin on the spawning grounds. An estimated 4.9 percent of spring-run chinook salmon (Oncorhynchus tshawytscha), 2.9 percent of summer-run chinook, 5.6 percent of fall-run chinook, 9.2 percent of silver salmon (O. kisutch), 0.6 percent of blueback (O. nerka), and 1.3 percent of steelhead trout (Salmo gairdnerii) caught by the gill-net fishery in 1957 were tuberculous. The incidence of tuberculosis in stocks of salmon returning to hatchery egg-collecting stations was generally much higher. No evidence of tuberculosis was found in silvers and fall chinook of probable natural origin on the spawning grounds. Fish heavily infected with tuberculosis were generally of smaller size than those lightly or non-infected.

Tuberculosis has been found in adult salmonid fishes upon their return from the sea and in young fish of these species held in fresh water for an extended period. Evidence has accumulated which indicates that the disease has been disseminated as a result of the fish-cultural practices used in hatchery propagation of these fish (Wood and Ordal, 1958). In order to understand more fully the factors influencing the occurrence and geographical distribution of tuberculosis and its possible effect on anadromous salmonid fishes in the streams of Oregon, a comprehensive survey was initiated in 1957.

METHODS

Four species of Pacific salmon including chinook (spring-, summer-, and fall-run) (Oncorhynchus tshawytscha), silver (O. kisutch), blueback (O. nerka), and chum (O. keta) and the steelhead trout (Salmo gairdnerii) were included in the survey. The spring, summer, and fall chinook salmon found in the Columbia River are identified as three general races. Salmon from the spring-run enter the river from January through May and migrate to the headwaters of the Columbia River and its tributaries. Summer-run chinook enter the river in June and July and spawn mainly in the upper Columbia and its tributaries, while fall-run chinook enter from August to December and spawn in the lower river tributaries of the Columbia as well as in the main stems of the upper Columbia and Snake Rivers. Adult fish were collected for examination from the commercial gill-net fishery landings on the Columbia River, at hatchery egg-collecting stations, and on the spawning grounds in areas where natural reproduction supports the run.

Usually no attempt was made to separate year classes in taking samples of fish for examination except, in the case of some hatchery samples, jacks (precocious males) were not included. Generally, in the streams of Oregon, the majority of fall chinook return to spawn in their third and fourth years; spring chinook in their fourth and fifth years; silver salmon in their third year; and blueback, chum, and steelhead in their fourth year.

The methods employed in the examination of all fish were essentially the same. After a

sample of fish to be examined was selected, the liver of each fish was removed and secured in an individual plastic bag. In most instances the livers were then frozen before transporting them to the laboratory, although some were taken to the laboratory in a fresh condition. Smears of liver tissue were prepared at the laboratory, stained by the Ziehl-Neelsen method, and examined for the presence of typical acid-fast bacilli. Fish were classified as tuberculous when typical acid-fast bacilli were found in the stained preparations within a five-minute search period under oil immersion. Each smear was classified according to the number of acid-fast bacilli observed. The classification system used was as follows:

Negative, no bacilli found during a five-minute search.

No. 1, only 1 to 20 bacilli found during a five-minute search.

No. 2, over 20 bacilli found during a five-minute search up to an average of less than 1 in many fields.

No. 3, about 1 to 24 bacilli on an average in each field.

No. 4, about 25 to 150 bacilli on an average in each field.

No. 5, more than 150 bacilli on an average in each field.

This system is a modification, for the purpose of simplification, of the rather awkward Gaffky scheme of rating smears for Mycobacterium tuberculosis outlined by Kolmer, Spaulding, and Robinson (1951). Certain errors are inherent in an arbitrary classification system such as the one outlined above. The area of the liver from which the smear was prepared was found, in a few instances, to make a difference of as many as two numbers in the system. There was usually close agreement, not over one number, between individuals reading a particular smear; the most serious error being between negative and number 1. It is apparent with the use of this system that a comparison of the degree of infection between groups of fish is subject to much less error than a comparison between individual fish.

COLUMBIA RIVER FISHERY

General

In order to obtain an estimate of the incidence of tuberculosis in fish entering the Columbia, it was necessary to rely on the commercial gill-net fishery. The landings of this fishery represented the only source of sufficient numbers of fish for the survey. Legislation has eliminated all except drift gill nets, dip nets, and sport gear. By far the bulk of the Columbia River catch is now taken in gill nets.

Commercial gill-net fishing takes place from the mouth of the Columbia to Bonneville Dam (fig. 1), a distance of approximately 140 miles. Chinook, silver, blueback, and chum salmon as well as steelhead trout are taken in the commercial fishery. The seasons open to the fishery in 1957 were as follows:

January 29 (noon) to March 1 (noon)
April 30 (noon) to May 27 (6 a.m.)
June 20 (6 a.m.) to July 15 (6 a.m.)
July 29 (6 p.m.) to August 26 (6 p.m.)
October 29 (6 p.m.) to November 29 (6 p.m.)

Closed weekends existed throughout the open seasons as follows:

May 1 to Aug. 1 Saturday (noon) to Sunday (6 p.m.)

Aug. 2 to Aug. 25 Friday (6 p.m.) to Sunday (6 p.m.)

Sept. 16 to Dec. 1 Friday (6 p.m.) to Tuesday (6 p.m.)

Whether the sampling of the landings furnished a reasonable accurate estimate of the incidence of tuberculosis in fish entering the river depended, in part, on whether the migrating adults were adequately sampled by the gill-net fishery. At the present time there are not sufficient data available to determine whether tuberculous fish are more readily taken than non-tuberculous fish by the gill-net fishery. Several sport fishermen, however, upon furnishing diseased tissues of fish to our laboratory have reported that these diseased fish did not "fight" like normal fish. In nearly all cases the tissues were heavily infected with tuberculosis. It is

suspected, therefore, that there might be a differential take of tuberculous fish since diseased fish, especially those heavily infected, are possibly not as able to free themselves from the nets as non-diseased fish. If such a differential existed in 1957, however, its importance is minimized when it is considered that an estimated 45 percent of the April and May spring-run chinook, 35 percent of the summer-run chinook, 45 percent of the fall-run chinook, 45 percent of the blueback, and 40 percent of the summer-run steelhead entering the Columbia were caught by the gill-net fishery. Since the bulk of the escapement through the fishery occurs during the closed seasons, the actual proportions of fish caught that were present in the river during the open seasons are probably much higher than those indicated above.

An influencing factor of possibly greater importance than a differential catch of tuberculous fish in obtaining an accurate estimate of the incidence of tuberculosis in fish entering the river is that fish for the survey could only be obtained during the open fishing seasons. Another factor of possible influence is that tuberculosis apparently influences the time of migration of chinook salmon, in particular, into the Columbia (Wood and Ordal, 1958). Tuberculous fall chinook have been found to enter the Columbia during almost any month of the year. The disposition of abnormally early migrants after they enter the river is generally unknown and it is quite possible that they are exposed to the fishery for a longer than normal period of time previous to entering the tributary streams. If so, these particular fish are probably sampled out of proportion to the normal migrants entering the river. More data are necessary before the degree to which these abnormally early fall chinook migrants affect the observed incidence of infection in chinook caught during the spring and summer seasons.

Due to the sampling of fish in the river only during the open fishing seasons, the results of the survey in 1957 are listed in this report as the incidence of tuberculosis in the landings of the gill-net fishery. In view of the above, this is the most accurate estimate available of the incidence of the disease in fish entering the Columbia in 1957.

Chinook salmon

The sampling of chinook salmon commenced during the week ending May 18, thus that portion of the spring run entering the river during the open fishing season in February and during the first two weeks of the season opening April 30 was not sampled. The data on tuberculosis in chinook are shown in table 1 and are divided to cover the three races. Weekly samples of varying size were obtained in each full week of open season. Livers were usually obtained from fish caught in all statistical zones (fig. 1) with the bulk of the sampling effort being concentrated on Zones I and II near the mouth of the river, the area where most of the chinook are usually taken. Since the number of fish caught and the size of the sample varied from week to week during the open seasons, the sampling data in table 1 are weighted by the number of fish caught during the week to give the estimated tuberculous number caught by week and by race.

It may be seen in table 1 that an estimated 4.9 percent of the spring-run chinook, 2.9 percent of the summer-run chinook, and 5.6 percent of the fall-run chinook taken by the fishery in 1957 were tuberculous. A direct comparison of the percentages is not thought to be entirely valid due to the previously mentioned migration of tuberculous fall chinook into the river during the spring and summer seasons. It may also be noted in table 1 that the comparatively small landings during the weeks ending August 3 and 10 are accompanied by higher than average rates of infection. Again in late October and November small landings are associated with comparatively high rates of infection. This would further indicate that the time of migration of many tuberculous chinook is somehow different than that of non-diseased chinook.

In table 8 are listed the number of chinook examined, by race, according to the relative abundance of acid-fast bacilli in the smears.

Liver samples were taken from a large portion of the marked fall chinook (fish marked as juveniles by excision of fins and/or maxillary bones) that were observed in the gill-net fishery landings during August to November by biologists of the Oregon Fish Commission and the Washington State Department of Fisheries. Data on marked

chinook that were examined are shown in table 2. Only double fin marks are included as single fin marks are often of questionable validity when taken in the fishery. Since data on the various marking experiments involved are not available, no comments can be made. However, there appears to be a great deal of variation in the incidence of tuberculosis between various groups of marked fish examined.

Silver salmon

The silver salmon enter the river in the fall months from August through December. Most spawn in the lower tributaries; a few early silvers spawn in some of the tributaries of the upper Columbia. Samples of the landings were obtained during each full week of the open seasons in which significant landings were made. Landings from all zones were sampled, however, 65 percent of the samples were obtained from Zones II and III. Approximately 82 percent of all landings in 1957 were from these zones.

The data in table 3 show the observed incidence of tuberculosis in silver salmon in 1957 by weekly periods and the estimated incidence for the entire run. As with the chinook, the sampling data were weighted by the number of fish caught to give the estimated number of tuberculous fish caught weekly, and for the entire run.

There is no evidence, to date, to suggest that tuberculosis affects the timing of silver salmon migration into the Columbia. This makes an analysis of the data in table 3 less complicated than that for the chinook in table 1. It may be seen in table 3 that the peak incidence of infection was observed in mid-October. If September 28 is used as an arbitrary division date between early- and late-run silvers, the incidence of infection observed in the early-run silvers (6.0 percent) appears significantly lower than that in late-run silvers (11.5 percent). Further, it may be seen in table 4 that if silver salmon caught in Zones I and II are separated from those caught in Zones III to V, the incidence of infection in those caught in the lower zones (I and II) appears significantly higher than those caught in the upper zones (III to V). These data indicate that the incidence of tuberculosis is higher in late-run silvers, which enter the lower tributaries of the Columbia to spawn, than it is in early-run silvers, which generally enter tributaries further upstream to spawn. It may be pointed out that the major effort of artificial propagation of silvers is concentrated on the lower tributaries. In view of the observed influence of fish-cultural procedures on the incidence of tuberculosis by Wood and Ordal (1958), artificial propagation is thought to account for the observed difference in the incidence of tuberculosis in early- and late-run silvers.

In table 8, the number of silver salmon examined are listed according to the relative abundance of acid-fast bacilli in the prepared smears. Using the arbitrary date of September 28, it may be seen that the degree of infection is much higher in the late-run than it is in the early-run silvers.

In table 5 are listed the fin-marked silver salmon recovered from the gill-net fishery landings in 1957 that were examined for tuberculosis. As with the marked chinook only double-fin mark recoveries are listed. It is a point of interest here that tuberculosis was not diagnosed in the fish that were fed the experimental diet (Ad-LV mark) while it was in a large portion of those that were fed the standard hatchery diet (Ad-RV mark) at the Sandy Hatchery. This will be discussed in a later section of the report dealing with the return of marked fish to the hatcheries.

Blueback

The blueback salmon enter the river in June and July and spawn in tributaries of the upper Columbia which have lakes in the vicinity of the spawning areas. Samples were collected each week of the fishing season in which significant landings were made. Landings from all zones were sampled during the season.

The data in table 6 show the observed incidence of infection in blueback salmon in 1957. As with the other species the sampling data are weighted by the number of fish caught to give the estimated number of tuberculous fish caught by week and for the entire season. The observed incidence of tuberculosis in this species is very low compared to that observed in chinook or silver salmon.

Table 1.--Incidence of Tuberculosis in Chinook Salmon Caught by Columbia River Gill-Net Fishery, 1957

Week Ending	Number of Fish Sampled	Percent of Sample Tuberculous	Number of Fish Caught	Estimated Number of Tuberculous Fish Caught	Estimated Percent of Tuberculous Fish in Run by Race
Spring:					
May 18	204	5.4	13,215	714	
25	280	4.6	17,019	882	
31 1/			2,165		
Total	484		32,399	1,596	4.9
Summer:					
June 22	162	1.9	27,414	521	
29	195	3.6	22,946	826	
July 6	127	3.1	13,151	408	
13	122	4.1	7,776	353	
20 1/			827		
Total	606		72,114	2,108	2.9
Fall:					
August 3	165	9.7	4,776	463	
10	145	13.8	9,163	1,264	
17	163	7.3	18,200	1,329	
24	258	4.3	85,556	4,019	
31 1/			7,899		
Sept. 21	165	6.1	16,639	1,015	
28	103	1.0	3,850	39	
Oct. 5	106	2.8	1,747	49	
12	141	5.7	1,543	89	
19	70	7.1	355	25	
Nov. 2 - 30	50	12.0	489	59	
Total	1,366		150,217	8,351	5.6

1/ Season open on first two days of week only; included with previous weeks sample.

Table 2.--Incidence and Degree of Tuberculosis Infection in Marked Chinook Salmon Caught by Columbia River Gill-Net Fishery, 1957.

Mark 1/	Origin 2/	Brood Year	Organization 3/	Number of Fish Examined by Relative Abundance of Acid-Fast Bacilli in Smears						Total	
				Neg.	1	2	3	4	5	Examined	Positive
D-RV	Little White Salmon R.	1953	F.W.L.S.	2	1		1	2		6	4
Ad-LP	Bonneville	1953	O.F.C.						1	1	1
Ad-RP	Willamette	1953	O.F.C.	1			1		1	3	2
	Bonneville	1954	O.F.C.		1				1	2	2
Ad-LV	Klickitat R.	1954	W.S.D.F.	31						31	0
Ad-RV	Klickitat R.	1954	W.S.D.F.	15						15	0
		1955	W.S.D.F.	1						1	0
An-LP	Wind R.	1953 or 1954	F.W.L.S.	2		1	1	1		5	3
An-RP	Little White Salmon R.	1953	F.W.L.S.	3	9	2	2			16	13
An-LV	Wind R.	1954	F.W.L.S.						1	1	1
An-RV	Spring Cr.	1953	F.W.L.S.	5	1					6	1

1/ Explanation of Marks: D - Dorsal fin; Ad - Adipose fin; An - Anal fin; LP - Left Pectoral fin; RP - Right Pectoral fin; LV - Left Ventral fin; RV - Right Ventral fin; LMax - Left Maxillary bone; RMax - Right Maxillary bone

2/ Area of experiment and/or release

3/ Organization sponsoring experiment: F.W.L.S. - Fish and Wildlife Service; O.F.C. - Oregon Fish Commission; W.S.D.F. - Washington State Department of Fisheries

Table 3.--Incidence of Tuberculosis in Silver Salmon Caught by Columbia River Gill-Net Fishery, 1957

Week Ending	Number of Fish Sampled	Percent of Sample Tuberculous	Number of Fish Caught	Estimated Number of Tuberculous Fish Caught	Estimated Percent of Tuberculous Fish in Run
August 3 - 31	164	4.3	1,875	81	
Sept. 21	179	4.5	10,499	472	
28	103	8.7	7,222	628	
Oct. 5	132	12.1	9,097	1,101	
12	134	11.9	10,349	1,232	
19	150	14.7	3,449	509	
26 1/			14		
Nov. 2	94	8.5	1,849	157	
9	77	5.2	687	36	
16 - 30	66	1.5	1,092	16	
Total	1,099		46,133	4,232	9.2

1/ Season open on first two days of week only; included with previous weeks sample.

Table 4.--Incidence of Tuberculosis, by Zones, in Silver Salmon Caught by Columbia River Gill-Net Fishery, 1957.

Week Ending	Number of Fish Sampled	Percent of Sample Tuberculous	Number of Fish Caught	Estimated Number of Tuberculous Fish Caught	Estimated Percent of Tuberculous Fish in Run, by Zones
Zones I and II: 1/					
August 3 - 31	108	3.7	1,030	38	
Sept. 21	72	5.6	8,018	449	
28	69	10.1	4,379	442	
Oct. 5	64	15.6	5,709	891	
12	62	24.2	6,337	1,534	
19	74	20.3	1,896	385	
Nov. 2	63	11.1	1,098	122	
9 - 30	66	6.1	969	59	
Total	578		29,436	3,920	13.3
Zones III, IV, and V: 1/					
August 3 - 31	56	5.4	845	46	
Sept. 21	67	3.0	2,481	74	
28	34	5.9	2,843	168	
Oct. 5	68	8.8	3,388	298	
12	72	1.4	4,012	56	
19	76	9.2	1,553	144	
26 2/			14		
Nov. 2	31	3.2	751	24	
9 - 30	36	0.0	810	0	
Total	440		16,697	810	4.9

1/ Samples from combined Zone II and Zone III landings not included

2/ Season open on first two days of week only; included with previous weeks sample.

Table 5.--Incidence and Degree of Tuberculosis Infection in Marked Silver Salmon Caught by Columbia River Gill-Net Fishery, 1957.

Mark 1/	Origin 2/	Brood Year	Organization 3/	Number of Fish Examined by Relative Abundance of Acid-Fast Bacilli in Smears						Total	
				Neg.	1	2	3	4	5	Examined	Positive
Ad-LV 4/	Sandy R.	1954	O.F.C.	25						25	0
Ad-RV 5/	Sandy R.	1954	O.F.C.	5	2	7	2			16	11
Ad-IMax 5/	Sandy R.	1955	O.F.C.		1					1	1

1/ For explanation of marks see Table 2.

2/ Area of experiment and/or release.

3/ Organization sponsoring experiment: O.F.C. - Oregon Fish Commission

4/ Fed experimental diet at hatchery.

5/ Fed standard hatchery diet.

Table 6.--Incidence of Tuberculosis in Blueback Salmon Caught by Columbia River Gill-Net Fishery, 1957

Week Ending	Number of Fish Sampled	Percent of Sample Tuberculous	Number of Fish Caught	Estimated Number of Tuberculous Fish Caught	Estimated Percent of Tuberculous Fish in Run
June 22	58	0.0	9,885	0	
29	240	1.25	30,581	382	
July 6	181	0.0	20,597	0	
13	104	0.96	4,882	47	
20 1/			123		
Aug. 1	1	0.0	51	0	
Total	584		66,059	429	0.6

1/ Season open on first two days of week only; included with previous weeks sample.

Table 7.--Incidence of Tuberculosis in Steelhead Trout Caught by Columbia River Gill-Net Fishery, 1957

Week Ending	Number of Fish Sampled	Percent of Sample Tuberculous	Number of Fish Caught	Estimated Number of Tuberculous Fish Caught	Estimated Percent of Tuberculous Fish in Run
June 29	120	1.7	9,390	160	
July 6	154	1.3	21,062	274	
13	122	1.6	19,078	314	
20 1/			546		
Aug. 3	153	0.7	3,814	27	
10	108	1.9	2,887	55	
17	168	0.0	3,515	0	
24	296	1.7	6,907	134	
31 1/			985		
Sept. 21	12	0.0	3,208	0	
28	30	0.0	564	0	
Oct. 5 - Nov. 30	58	1.7	355	6	
Total	1,221		72,539	970	1.3

1/ Season open on first two days of week only; included with previous weeks sample.

Table 8.--Degree of Tuberculosis Infection in Salmon and Steelhead Caught by Columbia River Gill-Net Fishery, 1957

Species	Season	Number of Fish Examined by Relative Abundance of Acid-Fast Bacilli in Smears						Total
		Neg.	1	2	3	4	5	
Chinook	May	460	10	2	3	6	3	484
	June-July	587	7	1	1	5	5	606
	Aug.-Nov.	1,274	20	14	15	27	16	1,366
	Total	2,321	37	17	19	38	24	2,456
Silver	To Sept. 28	422	13	7	3	1		446
	After Sept. 28	586	14	10	20	12	11	653
	Total	1,008	27	17	23	13	11	1,099
Blueback		580	3	1				584
Steelhead		1,206	6	3	1	2	3	1,221

Only one blueback with a double-fin mark was examined from the commercial catch in 1957. The marked fish was one of a group of 51,833 blueback of the 1953 brood that were reared at the Metolius River Hatchery of the Oregon Fish Commission, marked by excision of the adipose and left ventral fins, and liberated into Suttle Lake in September 1954. This one marked fish examined was tuberculous.

In table 8 the number of blueback examined are listed according to the relative abundance of acid-fast bacilli observed in the prepared smears. It may be seen that the degree of infection is relatively light compared with the other species.

Steelhead

The steelhead trout enter the Columbia in nearly every month of the year and spawn in practically all of the tributaries. The sampling of the species did not commence until June so only the so-called summer steelhead were examined. In general, summer steelhead pass through the lower river from June to October and are destined for tributaries of the upper Columbia. Winter steelhead, on which the major emphasis of artificial propagation is placed, pass through the lower river from November through May and spawn in tributaries of the lower river. Samples were taken in each full week of the open seasons in which significant landings were made. Landings from all zones were sampled during the survey.

The data on tuberculosis in steelhead trout caught by the gill-net fishery in 1957 are shown in table 7. As with the other species, these data are weighted by the number of fish caught to give the estimated number of tuberculous fish caught by week and by season. Compared with chinook and silver salmon, the estimated percentage of steelhead taken by the fishery that were tuberculous is much lower, although it is somewhat higher than that for blueback.

Only one double-fin marked steelhead caught by the fishery was examined in 1957. This was a fish marked by excision of the adipose and right pectoral fin. It is probable that this fish was of the 1953 brood and was one of 31,000 liberated by the Oregon Game Commission into the Deschutes River. It was not found to be tuberculous.

The number of steelhead examined are listed in table 8 according to the relative number of acid-fast bacilli observed in the prepared smears.

HATCHERY EGG-COLLECTING STATIONS

Adult fish returning to the various hatchery egg-collecting stations of the Oregon Fish Commission were sampled for tuberculosis in 1957. The locations of these hatcheries are shown in fig. 1. With few exceptions, the adults returning to the hatcheries include both wild and hatchery-reared fish. In most cases the proportion of each is unknown for any particular species at any particular hatchery.

A sample of 40 livers or more was obtained from fish of each species returning to the individual hatcheries; however, in some cases, a lesser number was taken due to the small number of adults appearing at the egg-collection stations or for other reasons. The samples, usually obtained by the hatchery personnel, were taken from the middle portion of the respective runs. Data included with each liver sample included the fork-length, sex, and species of the fish. Livers of marked fish were taken separately from the sample.

Five groups of fish were sampled more comprehensively. These were silver salmon returning to Klaskanine Hatchery; fall chinook returning to Bonneville Hatchery; and spring chinook, blueback, and steelhead returning to the Willamette (Oakridge) Hatchery. At these hatcheries, livers were taken in a random manner throughout the period of time that the fish were present at the egg-collecting stations. Two of these groups, blueback and steelhead, were of special interest in that they represented the first return of adults of these species to the Middle Fork of the Willamette River. The runs resulted from hatchery liberations of 1953 brood fish from the Willamette Hatchery.

Data on tuberculosis in the samples of salmon and steelhead returning to the various hatchery egg-collecting stations are shown in table 9. These data are listed by degree and incidence for each hatchery. The hatcheries located on tributaries of the Columbia River are listed separately from those located on coastal streams.

Data on marked fish returning to the various hatcheries are shown in table 10. Some single-fin marks, as well as double-fin marks, are included where the assignment of origin appears to be valid.

The data on several groups of marked fish originating from Oregon Fish Commission hatcheries are of interest. It may be noted in tables 5 and 10 that none of the 49 Ad-LV marked silvers caught by the fishery or returning to Sandy Hatchery were tuberculous. In contrast, 32 of 41 Ad-RV marked silvers caught by the fishery or returning to the hatchery were tuberculous. As young fish of the 1954 brood at Sandy Hatchery, both groups, made up of approximately 16,000 fish each, were fed the same diet for the initial 2-month feeding period. This initial diet contained untreated salmon viscera, beef liver, and meal. During the next 9 months, one group (Ad-RV) was fed the standard hatchery diet, which contained approximately 60 percent untreated salmon viscera. The other group (Ad-LV) was fed an experimental diet containing no salmon products. The difference in the incidence of tuberculosis between the two groups upon their return to the fishery and to the hatchery was considered by Wood (1958) to be strong evidence that tuberculosis in hatchery-reared silver salmon is greatly influenced by the fish-cultural practice of feeding untreated salmon products.

The experiment was repeated using 1955-brood silver salmon at the Sandy Hatchery. The diet during the initial 2-month feeding period and the standard hatchery diet (fed to fish marked Ad-LMax) again included untreated salmon viscera. The experimental diet (fed to fish marked Ad-RMax) did not contain salmon products. As can be seen in tables 5 and 10, 1 Ad-LMax marked silver and 4 Ad-RMax marked silvers returned as jacks (precocious males). One fish in each group was found to be tuberculous. The return of these two groups as adults in 1958 will furnish additional information on the feeding of untreated salmon products in the diet.

Another point of interest is that it may be noted in tables 2, 9, and 10 that the incidence of tuberculosis in marked chinook originating from Bonneville Hatchery is much higher than that noted in unmarked chinook returning to Bonneville Hatchery. This was noted previously by Wood and Ordal (1958) and was thought to be primarily the result of marked chinook being reared at the hatchery for a longer period of time than the unmarked. Chinook of the 1952, 1953, and 1954 brood years that were marked were held at the hatchery longer than chinook of the same brood years that were released unmarked. In addition, it may be noted in table 10 that marked chinook originating from several other hatcheries entered Tanner, Eagle, and Herman Creeks. Straying of chinook originating in other tributaries into streams in the Bonneville area may account for part of the difference in the observed incidence of infection between marked and unmarked chinook.

Wood and Ordal (1958) previously noted that steelhead heavily infected with tuberculosis were generally smaller than those lightly or non-infected. A similar relationship was noted in other species examined during the 1957 survey. In table 11 are shown comparisons of average fork lengths of lightly (or non-infected) and heavily infected fish. Lightly or non-infected fish were those whose liver smears were rated 1, 2, or negative according to the arbitrary rating system discussed earlier. Heavily infected fish were those whose liver smears were rated 3, 4, or 5. Certain criteria were used in selecting the groups for size comparison in table 11. Only groups that consisted of fish of the same age and for which there were at least 20 fish, by sex, in both the heavily and lightly infected (or non-infected) categories were included.

On the basis of Student's t test, the fork lengths of heavily infected fish were significantly different (at the 5 percent level) than those of lightly or non-infected fish, except for three groups. Those not found to be significantly different were Ad-RP marked falls from Bonneville Hatchery and both male and female blueback from Willamette Hatchery.

Table 9.--Incidence and Degree of Tuberculosis Infection in Salmon and Steelhead Returning to Hatchery Egg-Collecting Stations, 1957

Hatchery	Species	Number of Fish Examined by Relative Abundance of Acid-Fast Bacilli in Smears						Total		Percent Tuberculous
		Neg.	1	2	3	4	5	Examined	Positive	
Columbia River:										
Klaskanine	Fall Chinook				2			2	2	100
	Silver	12	27	55	66	41	46	247	235	95
Big Creek	Fall Chinook	7	2	3	3	7	6	28	21	75
	Silver	5	4	8	15	9	9	50	45	90
	Chum	45					1	46	1	2
North Santiam	Spring Chinook	30	5	6	3		4	48	18	38
South Santiam	Spring Chinook	33	2	3	4	3		45	12	27
McKenzie	Spring Chinook	71	3	1	3			78	7	9
Willamette (Oakridge)	Spring Chinook	268	125	100	62	35	15	605	337	56 1/
	Steelhead	1	27	22	15	21	20	106	105	99
	Blueback	9	15	38	25	18	3	108	99	92
Sandy	Chinook 2/	71	18	9	39	19	17	173	102	59
	Silver	34	7	18	1		2	62	28	45
Bonneville	Fall Chinook	216	34	18	30	42	41	381	165	43
Eagle Creek 3/	Fall Chinook	37	3	4	6	6	6	64	25	40
Ox Bow Springs	Fall Chinook	26	3	4	4	3	10	50	24	48
Metolius	Spring Chinook	14	1		3	2		20	6	30
Coastal:										
Nehalem	Silver			3	4	3	7	17	17	100
Trask	Spring Chinook		1	2	5	18	29	55	55	100
	Fall Chinook	3	1	1		1	2	8	5	63
	Silver	1		1		5	2	9	8	89
Siletz	Silver	2	1	4		3		10	8	80
Alsea	Silver	3	2	4	13	7	11	40	37	93
South Coos	Silver	1	3	3	12	10	3	32	31	97

1/ By weighting samples throughout season, a calculated 59 percent of the run was tuberculous.

2/ Spring chinook and fall chinook not separated.

3/ Egg-collecting station for Bonneville Hatchery.

Table 10.-- Incidence and Degree of Tuberculosis Infection in Marked Salmon Returning to Hatchery Egg-Collecting Stations, 1957.

Hatchery	Species	Mark 1/	Brood Year	Origin	Number of Fish Examined by Relative Abundance of Acid-Fast Bacilli in Smears: Neg.	1	2	3	4	5	Total Examined	Total Positive
Big Creek	Fall Chinook	Ad	1953 and 1954	Gnat Cr. Klaskanine R.				4	3	2	9	9
		An	1954	Elokomin R.	4	1	1				6	2
		RV	1954	Klickitat R.	2						2	0
		Ad-LV	1954	Klickitat R.	1	1					2	1
	Silver	Ad-LV	1955	Big Creek	32	19	3	1			55	23
		Ad-RV	1955	Big Creek	6	6	6				18	12
Willamette 2/ (Oakridge)	Spring Chinook	Ad-RV	1953	Willamette R.	8	22	12	12	8	5	67	59
Sandy	Spring Chinook	Ad-LV	1953	Sandy R.	2				1	1	4	2
	Silver	Ad-LV 3/	1954	Sandy R.	24						24	0
		Ad-RV 4/	1954	Sandy R.	4	6	9	5	1		25	21
		Ad-RMax 3/	1955	Sandy R.	3	1					4	1
Bonneville 5/	Fall Chinook	Ad-RV	1952	Bonneville					1		1	1
		Ad-LP	1953	Bonneville						2	2	2
		Ad-RP	1954	Bonneville	2	10	14	18	11	9	64	62
		Ad-LV	1954	Klickitat	8						8	0
			1955	Klickitat	1						1	0
Bonneville	Fall Chinook	Ad-RV	1954	Klickitat R.	5						5	0
			1955	Klickitat R.	2						2	0
		An-LP	1953 and 1954	Wind R.			1	1			3	3
		An-RP	1953	Little White Salmon	4	1	3	3			11	7
		An-RV	1953	Spring Creek	1						1	0

1/ For explanation of marks see Table 2.

2/ Includes 12 fish returning to McKenzie River.

3/ Fed experimental diet at hatchery.

4/ Fed standard hatchery diet at hatchery.

5/ Fish taken during egg-collecting operations on Tanner, Eagle, and Herman Creeks.

Table 11.--Comparison of Average Fork Lengths of Lightly and Heavily Infected Salmon and Steelhead Returning to Hatchery Egg-Collecting Stations, 1957.

Species	Hatchery	Sex	Brood Year	Lightly Infected 1/		Heavily Infected	
				Number of Fish	Average Fork Length (in.)	Number of Fish	Average Fork Length (in.)
Spring Chinook (Ad-RV mark)	Willamette	Male	1953	33	29.3	20	26.8
Steelhead	Willamette	Male	1953	21	28.1	31	26.8
		Female	1953	29	27.0	25	25.9
Blueback	Willamette	Male	1953	27	20.8	20	20.8
		Female	1953	35	19.8	26	19.5
Fall Chinook (Ad-RP mark)	Bonneville	Male	1954	23	21.5	36	20.6
Silver	Klaskanine	Male	1954	51	29.0	74	27.1
		Female	1954	32	27.0	72	26.1

1/ Includes a small number of non-infected fish.

NATURAL-REARED SALMON

In order to compare the incidence of tuberculosis in wild fish with that in hatchery-reared fish, a small number of fish of likely natural origin were examined in 1956 and 1957. Areas where adult fish of likely natural origin could be obtained in Oregon proved to be somewhat limited. It cannot be safely assumed that the adult fish in a particular stream are of natural origin because no hatchery-reared fish were released there, since hatchery-reared fish are widely distributed and the homing instinct of the salmon is not an infallible one.

Two particular areas presented the best apparent opportunity to obtain significant numbers of wild fish for examination. These were Tenmile Lakes for silver salmon and the Snake River in Idaho for fall chinook. Tenmile Lakes (fig. 1) support a run of silver salmon generally in excess of 50,000 yearly. Except for a small number of marked fish planted in 1956, no liberations of hatchery-reared salmon have been made in Tenmile Lakes in recent years. Possible straying of hatchery-reared fish from other streams into Tenmile Lakes are probably "diluted" to a great extent by the large number of silvers migrating naturally into Tenmile Lakes.

It is highly probable that fall chinook salmon spawning in the main stem of the Snake River in the area below Swan Falls Dam (see fig. 1) are exclusively of natural origin. No liberations of hatchery-reared fall chinook have been made in the Snake River, in fact, the nearest tributary of the Columbia where liberations of hatchery-reared fall chinook are made is located several hundred miles downstream from Swan Falls.

The results of the examination of salmon from Tenmile Lakes and the Snake River are shown in table 12. Silver salmon from Tenmile Lakes were obtained on the spawning grounds of the tributary streams in December 1956. Most of the fall chinook from the Snake were obtained on the spawning grounds in the vicinity of Swan Falls Dam in November 1957; an additional small number was obtained from anglers in the vicinity of Brownlee Damsite in September and October 1957.

As can be seen in table 12, no evidence of tuberculosis was found in these two groups of wild fish. On the basis of these data it may be anticipated that tuberculosis is a comparatively rare event among stocks of salmon of natural origin that are removed from areas where hatchery-reared fish are liberated. In areas where hatchery-reared fish are liberated the possibility must be considered that the disease may become established in natural populations as a result of the upstream migration and spawning of tuberculous adult salmon originating in hatcheries. It is obvious that further work must be done in order to establish evidence on this particular point.

LITERATURE CITED

Kolmer, J.A., E.H. Spaulding, and H.W. Robinson
1951. Approved Laboratory Technic. Fifth edition. Appleton-Century-Crofts, Inc., New York, xii 1, 180 pp.

Wood, J. W.
1958. Tuberculosis in Pacific salmon and steelhead trout. Master's Thesis, University of Washington, 75 pp.

Wood, J. W., and E. J. Ordal
1958. Tuberculosis in Pacific salmon and steelhead trout. Fish Commission Oregon., Contr. No. 25

Table 12.--Incidence of Tuberculosis in Natural-reared salmon examined in 1956 and 1957

Species	Year examined	Location	Number of fish examined	Number of fish positive
Silver	1956	Tenmile Lakes	184	0
Fall chinook	1957	Snake River (Swan Falls)	104	0
		(Brownlee Damsite)	13	0

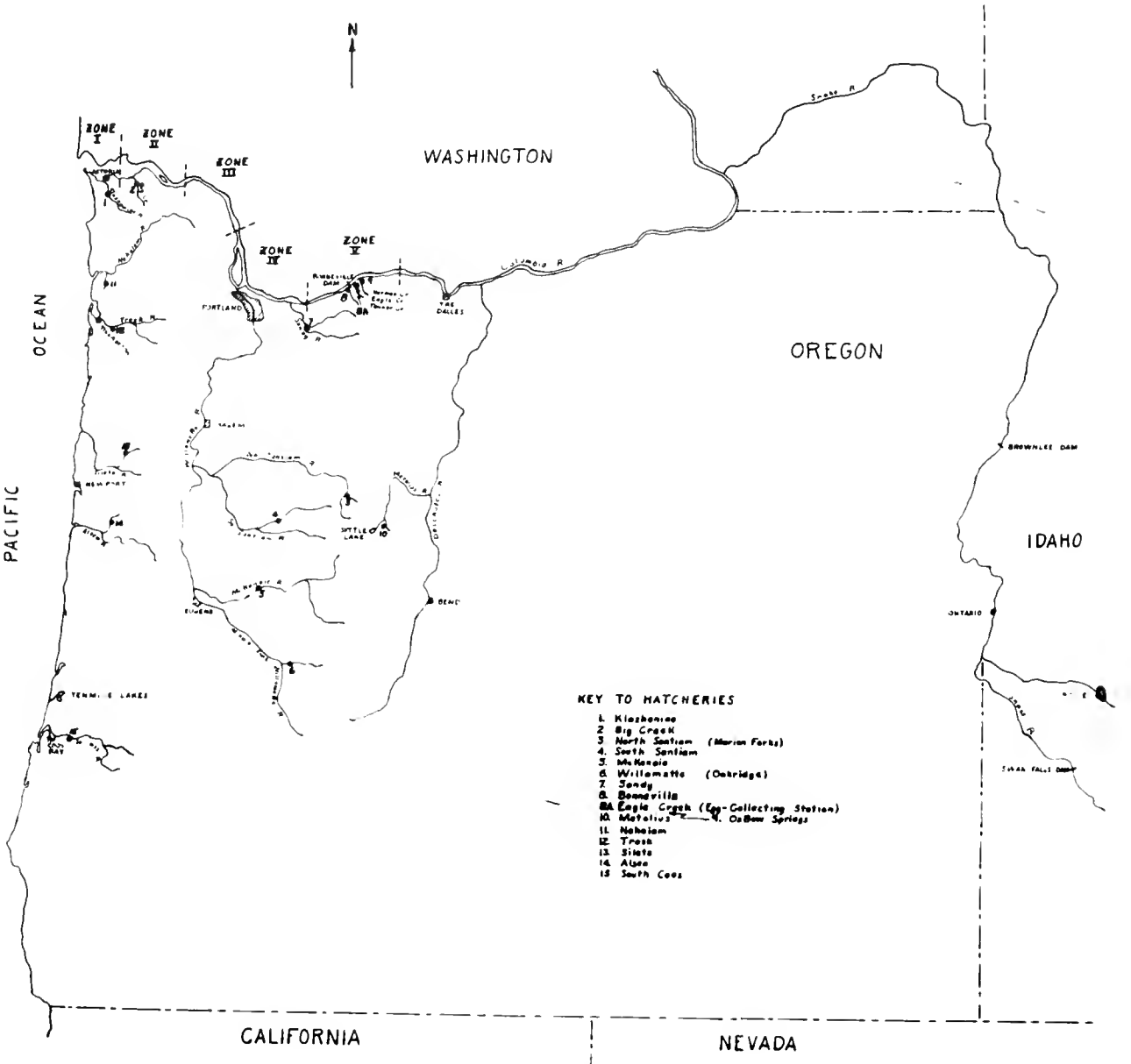

Figure 1. Area of Survey

INT.DUP..D.C.60-67066

MBL WHOI Library - Serials
5 WHSE 01460